Review
Allergy Asthma Immunol Res. 2015 November;7(6):525-533.
http://dx.doi.org/10.4168/aair.2015.7.6.525
pISSN 2092-7355 • eISSN 2092-7363

AAIR
Allergy, Asthma & Immunology Research

Models of Respiratory Infections: Virus-Induced Asthma Exacerbations and Beyond

Sara Saturni,[1] Marco Contoli,[1] Antonio Spanevello,[2] Alberto Papi[1]*

[1]*Section of Respiratory Medicine, University of Ferrara, Ferrara, Italy*
[2]*Department of Respiratory Diseases, Fondazione Maugeri, Tradate, University of Varese, Italy*

Respiratory infections are one of the main health problems worldwide. They are a challenging field of study due to an intricate relationship between the pathogenicity of microbes and the host's defenses. To better understand mechanisms of respiratory infections, different models have been developed. A model is the reproduction of a disease in a system that mimics human pathophysiology. For this reason, the best models should closely resemble real-life conditions. Thus, the human model is the best. However, human models of respiratory infections have some disadvantages that limit their role. Therefore, other models, including animal, *in vitro*, and mathematical ones, have been developed. We will discuss advantages and limitations of available models and focus on models of viral infections as triggers of asthma exacerbations, viral infections being one of the most frequent causes of exacerbating disease. Future studies should focus on the interrelation of various models.

Key Words: Respiratory tract infections; theoretical models; animal models; *in vitro* diagnostic devices; mathematical computing

INTRODUCTION

Respiratory infections are important clinical conditions because of their diffusion and also because of their potential severe expression and clinical consequences. For these reasons, in the past decade, many studies have deeply investigated the mechanism of respiratory infections to gain information for more effective treatment/prevention of these clinical events. Many different models have been developed. A model is a system that mimics the complexity of relationship between microbes and the host's defenses. This can be reproduced *in vivo* in humans and animals, and also *in vitro*. Moreover, some mathematical models have been created, with the aim of describing, in formulas, the principles of respiratory infections and their diffusion. Each model has advantages and limitations, which are described in this review. Among various models here considered, we will detail the models of viral infections causing acute clinical worsening of obstructive lung diseases, and in particular asthma and obstructive lung diseases, and in particular asthma and chronic obstructive pulmonary disease (COPD) exacerbations.

We will start describing the experimental setting that is considered the optimal modeling condition, *i.e.* human models, and then consider conditions more imperfect progressively less closely reproducing the complexity of human wild infections. The integration/association of different modeling will be finally considered.

Human models

Since a model endeavored to reproduce human pathophysiology, human models are most accurate. Clearly, the main advantages of human models include the possibility to evaluate the role of genetics, the exact reproduction of anatomical and physiological characteristics of the respiratory tract, and the possibility of direct analysis of inflammatory and immune responses, including pulmonary host defenses. Currently available human models are limited, for obvious safety and ethical reasons, to few viral infections of the respiratory tract.

DeVincenzo *et al.*[1] described a human model of respiratory syncytial virus (RSV) infection. The rationale of their work is based on the results of *in vitro* and animal models, which portray RSV disease severity as linked more to immunologic responses than to viral load.[1] However, in contrast to these premises, they demonstrated that, in humans, RSV infection symp-

Correspondence to: Alberto Papi, MD, Section of Respiratory Medicine, University of Ferrara, Via Savonarola 9, 44121 Ferrara, Italy.
Tel: +39 0532 236908; Fax +39 0532 210297; E-mail: ppa@unife.it
Received: February 25, 2015; Accepted; April 6, 2015
• There are no financial or other issues that might lead to conflict of interest.

toms depend on viral load and are not related to immunologic responses.[1] The study suffers from some limitations. In particular, the model reproduced a mild infection in adults, while usually RSV causes a severe disease in children. These aspects could be considered intrinsic limits of human models and are linked to ethical considerations, which do not allow the reproduction of severe infections (*e.g.*, bacterial infections) or the inclusion of children. For these reasons, in the young population, pneumonia caused by *Mycoplasma pneumoniae* have been retrospectively studied.[2]

Human models of virus-induced asthma exacerbations

There is much evidence of the causal role of respiratory viruses in asthma exacerbations.[3] Respiratory viruses have been detected in >80% of asthma exacerbation cases of children[4] and in 44% in adults,[5] and rhinoviruses (RVs) is most common, accounting for 60%-66% of viral cases.[3] A strong epidemiological association of RVs with asthma exacerbations is sided by evidence supporting that RVs spread not only in the upper but also in the lower airways, inducing inflammation.[6] Both infected bronchial and pulmonary epithelial cells produce inflammatory factors that enhance airway inflammation and obstruction.[7] In a human model, RV infection in asthmatics induced the most typical characteristics of exacerbating asthma: lung function impairment, worsening symptoms, increased bronchial reactivity, and eosinophilic airway inflammation.[8] The exacerbation severity was related to immunological changes, in particular to deficient interferon (IFN)-γ and interleukin (IL)-10 and to increased IL-4, IL-5, and IL-13.[8] The viral load related to clinical and functional parameters in asthmatic patients, but not in healthy controls, revealing relationships between viral infections and severity of asthma exacerbations.[8]

Bronchial mucosal biopsies derived from bronchoscopies of experimental RV infections in asthmatics revealed an increase in epithelial eosinophils.[9] Moreover, during the RV colds there are a burden in submucosal lymphocytes and a peripheral blood lymphopenia, both related to the increase in histamine responsiveness.[9] A further model of RV-induced asthma exacerbations demonstrated a more important increase in neutrophils and CD681 macrophages in bronchial mucosa of asthmatics compared to healthy controls. This inflammation was related to viral load and correlated with a decrease in lung function, underlying a role of RV-induced inflammation in the severity of asthma exacerbations.[10]

Human models of virus-induced COPD exacerbations

Similarly, a human model demonstrated a connection between viral infections and COPD exacerbations.[11] Since it is very difficult to examine naturally occurring COPD exacerbations, in order to better understand and study the mechanisms of virus-induced COPD exacerbations, experimental RV infection in COPD patients has been developed.[11] The experimental RV16 infection produced typical COPD exacerbations' clinical features associated with worse airflow obstruction and respiratory symptoms/inflammation (both local and systemic) in COPD patients compared to controls.[11] In COPD patients, as in asthmatics, exacerbation severity related to immunologic alterations, such as an increase in neutrophils.[11]

Symptoms and inflammatory markers were associated with viral load.[11] Interestingly, viral load was higher, and IFN production was lower in COPD patients compared to healthy controls.[11] Thus, impaired IFN-γ production and neutrophilic inflammation may play an important role in RV-induced COPD exacerbations.[11]

Even if human models are one of the truest representations of infectious respiratory diseases and offer answers to many clinical questions, they have many weak points. In addition to those already described, like ethical limitations, further disadvantages of human models consist of poor adherence to therapy and confounding factors, such as comorbidities, that are common in real life but do not contaminate the "sterile control" conditions of an experimental model. Moreover, human models are often difficult to manage, requiring particular facilities that are available only in a few specialized centers. For these reasons, until now other models have been more extensively used.

Animal models

Animal models of respiratory infections have been widely studied. There are many models, and each has some advantages and disadvantages; thus, the choice of model is based on the question that is being examined (Table 1). For example, chimpanzees have anatomic, genetic, and immunological similarities with humans, so their use is essential in the development of vaccines.[12] Unfortunately, chimpanzees are expensive, are difficult to manage, and involve emotional aspects and ethical implications.[12]

Instead, cattle are used for modeling respiratory infections because they are the natural host of *Mycobacterium tuberculosis bovis* and usually develop a disease very similar to the human disease, so they have provided important information for tuberculosis vaccine development.[13] However, their most important disadvantage is also their largest limitation: they are affected by a specific *Mycobacterium* (*M. bovis*) that is similar to, but not the same as, the main human pathogen (*M. tuberculosis hominis*).[13] Moreover, they require a large amount of space and expensive equipment.[13]

Sheeps have been used to evaluate the role of infections in the development of the respiratory system and in lung function because these aspects are easily measurable in these animals.[12] Unfortunately, limited molecular tools and difficult handling limit studies on such animals.[12]

Rabbits and guinea pigs are easy to handle and particularly useful for studies examining the role of the immune system.[13] Nevertheless, high costs, a lack of suitable reagents, and diffi-

Table 1. Role of animal models*

Advantages	Limits	Answer on
Immunologic features	Expensive	Anatomic role (chimpanzees)
Genetic manipulation	Logistically difficult	Role of immune system (chimpanzees, rabbits, guinea pigs)
Availability of reagents	Ethical implications	Infection effect on lung function (sheeps)
Pulmonary-function tests feasibility	Emotional burden	Vaccines studies (cattle and *M. bovis* vaccine)
Relevant sample size	Immunologic differences	Genetic role by genetic manipulation (mice)
The smallest are easy to handle	The largest are difficult to handle	
The largest have anatomic features similar to humans	The smallest have huge difference from humans	

*describes the advantages and disadvantages of animal models, from the most similar to humans, but least used, to the most common, but more dissimilar to human conditions.

cult genetic manipulation limit their use.[13] Cotton rats are used in studies on viral respiratory infections because they are semi-permissive for viral replication and allow the evaluation of high viral levels.[12] Nevertheless, they are quite fragile and difficult to handle.[12] Certainly, the most commonly used animal models have been mice. Even if they have important immunologic and anatomical differences from humans, they are easy to house and handle, and these quantities permit a relevant sample size. These features, together with the increased availability of reagents and, above all, the wide possibility of genetic manipulation, support their extensive use.[12]

Animal models of virus-induced asthma exacerbations

Many small-animal models of RV infection have been attempted and failed, probably because among known RV serotypes, the majority (90%) use human intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor and do not bind mouse ICAM-1.[14] However, a minor RV group (10%) use a different adhesion molecule and can bind the mouse equivalent.[14] Using this minor-group of RV has been possible to create a mouse model of asthma exacerbation caused by RV infection.[14] An important study evaluated 3 mouse models of RV infections in BALB/c mice, in transgenic BALB/c mice with a mouse-human ICAM-1 chimera, and in a mouse model of asthma (BALB/c mice sensitized to ovalbumin [OVA] challenged with OVA or PBS), inducing exacerbation.[14] These animal models induced inflammatory changes, such as increases in mucins and some cytokines (IL-4 and IL-13 in the mouse model of asthma), which reproduce humans' responses, so they can be potentially useful in the study of both infectious and allergic conditions, as asthma exacerbations triggered by colds.[14] Another animal model, using OVA-sensitized and -challenged mice (which reproduce allergic airway inflammation), showed that RV infection induced type 2 cytokine production from airway macrophages, including eotaxin-1, IL-4, and IL-13.[15] These molecules increased eosinophilic inflammation and airway hyperresponsiveness.[15] The IL-4 signaling pathway is fundamental in regulating macrophage activation and the consequent pattern in the mouse model of RV-induced asthma exacerbation.[16] Indeed, without IL-4/IL-13 signaling, RV infection produces type 1 cytokine production, with subsequent increases in neutrophils; instead, in the presence of IL-4/IL-13 signaling, RV infection produces type 2 cytokine production from macrophages, with subsequent eosinophilic inflammation.[16] Interestingly, different viral infections have different consequences in mouse-models of asthma exacerbations. RSV infection in the house dust mite (HDM) mouse model of asthma increases all inflammatory cell types in BAL, but reduces eosinophils, and does not increase cytokines, while influenza infection produces an increase in BAL lymphocytes, neutrophils, IFN-γ, IL-1β, IL-4, IL-5, IL-10, and IL-12.[17] Moreover, infection by RSV reduces steroid sensitivity, while influenza infection is sensitive to this treatment, revealing novel pharmacological challenges for asthma exacerbation therapy.[17] Mouse models have also been able to reproduce corticosteroid-resistant asthma exacerbations, by using the relevant aeroallergen HDM and the viral mimic poly(I:C) (viral mimetic/toll-like receptor 3 [TLR3] agonist).[18] The inflammation enhanced in this model is characterized by neutrophils, without any increase in either Th2 cytokines or eosinophil chemoattractant, and is insensitive to oral prednisone therapy.[18] Thus, this model may provide a useful tool to better understand mechanisms of severe asthma exacerbations and to explore a novel therapeutic target.[18]

Animal models of virus and bacteria-induced COPD exacerbations

Many different approaches have been explored in order to reproduce COPD features in animals: exposure to noxious inhalants (usually cigarette smoke), use of tissue-degrading proteinases (*i.e.* elastase), and genetic manipulation.[19,20] The advantage of cigarette smoke-induced models is the use of the most important COPD causative agent. Nevertheless, in mice, even long-lasting exposure to this agent is able to induce only mild respiratory problems,[19,21] while use of proteinases induces more

severe features reproducing also advanced COPD.[20]

These animal models are particularly useful to study and describe COPD exacerbations and their mechanisms and causes, including viral and bacterial respiratory infections.[21]

In a mouse model of COPD, RV infection induced airway inflammation and impaired production of IFNs compared to controls.[22] In another cigarette smoke-induced guinea-pig model of COPD, latent adenoviral infection produced an increase in lung volume and a reduction in the surface/volume ratio, typical of emphysema lung destruction.[23] In some cigarette smoke-induced mouse models of COPD, mice were also infected with bacteria, such as *Haemophilus influenzae* or *Pseudomonas aeruginosa*, with subsequent increased pulmonary inflammation and clinical symptoms.[24] Instead, an elastase-treated mouse model of COPD showed a reduction in ICAM-1 in airway epithelium, suggesting that this impairs bacterial clearance.[25] Other studies will be necessary to assess similarities and discrepancies among bacterial flora of animal and human airways, which could potentially affect usability of bacteria-induced COPD exacerbation models.[20]

Animal models of pneumonia

Studies on mouse models are not limited to viral and bacterial common infections, but also to complicated infections like pneumonia. Animals can be manipulated in different ways to investigate various aspects of diseases. For instance, immunological insights into pneumonia have been obtained by photon-irradiation (non-lethal doses) of mice then challenged intratracheally with *Klebsiella pneumonia*.[26] These mice developed bronchopneumonia, and the lung inflammatory cellular reaction was inversely related to the irradiation dose, while Gram-negative amount (in colony-forming-unit) and mortality were directly related to the irradiation dose.[26] Interestingly, the irradiation mimicked immunodepression conditions providing us with an animal model of hospital-acquired pneumonia.[26]

Marquette *et al.*[27] developed a model of experimental spontaneous pneumonia in mechanically ventilated piglets. Even though these pneumonia infections were due mainly to common airway colonizing microorganisms,[27] while the majority of human ventilator-acquired pneumonia (VAP) infections are due to *Pseudomonas aeruginosa*,[28] this model provided vast insights into the pathophysiology of pneumonia. In order to obtain a correct model of VAP, Luna *et al.*[29] intubated piglets, treated them with mechanical ventilation, and induced pneumonia by direct inoculation with *Pseudomonas aeruginosa*. Even if this model did not reproduce the common pathophysiological sequence of events leading to VAP, this study gave important information about local and systemic responses in VAP, different patterns of mechanical ventilation during lung infections, and responses to antimicrobial therapy. An obvious limitation of this as of any "pure" animal model is that animals do not have comorbidities, which are common in ventilated patients and play a relevant role in the overall clinical condition and evolution/prognosis.

The immune/inflammatory airway responses are very important to the clinical outcome of the infection. Unfortunately, they cannot be exactly reproduced in animal models. Thus, in order to overcome this limitation, humanized models have been developed. Humanized mice are immunodeficient mice engrafted with functional human cells, tissues, and immune systems that are used to study human inflammatory defences.[30]

Respiratory epithelium has been successfully engrafted and reconstructed in mice.[31] Several applications, including the study of host-pathogen interactions, have been described. In particular, a study on humanized mice demonstrated that *Staphylococcus aureus* adhering to human airway epithelial cells is strongly linked to fibronectin.[32] Indeed, a FN-binding protein-deficient strain of *S. aureus* had a 5-fold lower adherence level to airway epithelial cells compared to the parental strain.[32] The study also showed that 97% of *S. aureus* clinical strains, isolated from the airway secretions of Cystic Fibrosis (CF) patients and patients with nosocomial pneumonia, possessed the FnBP.[32] The strains from patients affected by nosocomial pneumonia have higher FN-binding capacity than did those from CF patients. These results suggested a major role of FnBP in the development of pneumonia and a role also in colonization of airways by *S. aureus* in patients affected by CF.[32]

Even though humanized mice are very interesting and useful animal models that open new research possibilities for the future, they do have disadvantages. Some limitations include a lack of HLA molecules required for appropriate T-cell selection, a lack of appropriate HLA APCs, and species-specificity of growth factors and other molecules,[33] low levels of humoral immune responses[34] and residual innate immunity of the host.[30] Thus, further studies are needed before these models can be extensively used.

In vitro models

In vitro studies are important research tools in the field of respiratory infections. They have many advantages, including being quite inexpensive, easily controlled, well standardized, and not being influenced by comorbidities (Table 2). Moreover, they permit answers to be quickly obtained and reduce the exposure of animals and humans to potentially toxic or ineffective drug therapy.[35]

In vitro models are the first step to study a respiratory infection mechanism. They consist in a basic environment, with a controlled setting, which simplify the system to be studied. They allow us to evaluate the role of every single element/condition in order to clarify the pathway that leads to respiratory infections. After this first step, the interrelationship between individual elements can be studied in a complex setting as an animal/human model. A more complex setting is represented by the ex-vivo models, which consist of human samples that are

Table 2. Role of *in vitro* models*

Advantages	Limits	Answers on
Not influenced by comorbidities	Unable to evaluate host defenses	Gene role
Inexpensive	Unable to evaluate inoculum effect	Pathogenetic mechanisms
Easily controlled	For pathogens more virulent *in vivo*	Molecular mechanisms
Quick answer	Unable to evaluate drugs distribution	Therapies
Well standardized		

*lists the roles of *in vitro* models describing their positive and negative aspects.

infected *in vitro*.

In vitro models of RV infection

In vitro studies have been very important in studying which are molecular/inflammatory mechanisms of RV infection on the respiratory tract. They were able to demonstrate that RV increases airway inflammation, inducing the secretion of proinflammatory cytokines like IL-1β and IL-6.[3,36,37] Furthermore, models also revealed that, among proinflammatory mediators, RV infections induced TNF-α[36] which amplifies the viral infection because TNF-α is able to increase vulnerability of bronchial cells to RV.[3,37] Moreover, *in vitro* studies also showed that RV increases intracellular oxidant production, enhancing the production of inflammatory interleukins.[3,38] These virus-induced inflammatory mediators are reduced by β_2-agonists and glucocorticoids, as revealed by another *in vitro* study which evidenced the synergic effect of the combined therapy.[3,39] *In vitro* studies also showed that corticosteroids reduce RV-induced ICAM-1 upregulation in bronchial epithelial cells.[40]

Together with *in vitro* studies, *ex vivo* studies has also been extremely important in revealing immunologic mechanisms involved in respiratory RV infections. A defective innate immune response has been found in asthmatic primary bronchial epithelial cells and alveolar macrophages expressed as deficient interferon induction after an experimental infection. This impairment was related to increased viral replication and more severe clinical expression of the infection (exacerbation) severity in asthmatic subjects.[41] Similarly, considering acquired immunity, *ex vivo* data on peripheral blood mononuclear cells (PBMC), exposed to RV, revealed that asthmatics have a defective type 1 and a higher type 2 response to RV infection compared to healthy controls.[42] Indeed, RV induced the production of inflammatory mediators in both groups of patients, but IFNγ and IL-12 levels were lower, while IL-10 levels were higher in asthmatics than in controls, and IL-4 is induced only in asthmatics.[42]

Moreover, it has recently been shown that RV infection of bronchial and nasal epithelial cells cocultured with eosinophils results in the impaired innate immune response and the enhanced inflammatory cascade.[43] These *ex vivo* data suggest a negative interplay between eosinophilic inflammation (a hallmark of asthmatic airway inflammation) and the outcomes of RV infection.[43]

An *in vitro* study on nasal polyp epithelial cells also demonstrated that RV-16 increases mucin production in the upper respiratory tract and that this secretion increases even more if there is a co-stimulation with fungi and eosinophils.[44] It would be interesting to study if these respiratory infections also have the same effects on the lower respiratory tract, potentially exacerbating the inflammatory process.

Recently, the effect of viral infection on the mechanism of action of glucocorticoids, the mainstream of asthma therapy, has been investigated. Indeed, despite optimal treatment, asthma exacerbations still occur. Cultured primary human bronchial or transformed (A549) respiratory epithelia were infected with RV16 and then exposed to dexamethasone.[45] This approach revealed that RV infection reduces binding of the glucocorticoid receptor to glucocorticoid response elements in airway epithelial cells, due to impaired GR nuclear translocation, *i.e.* it impairs the limiting step of the mechanism of action of glucocorticosteroids inducing a sort of steroid resistance.[45] RV-16 infection alters all outcomes depending from dexamethasone, including inhibition of CXCL8 release induced by IL-1β and expression of genes for mitogen-activated protein kinase phosphatase 1.[45] RV-16 infection induces nuclear factor kB activation and GRα phosphorylation, so the proinflammatory pathways JNK and NF-kB are both activated by RV and are key elements in RV-induced steroid insensitivity.[45] However, inhibitors of IKK2 and JNK are able to prevent these consequences restoring dexamethasone effects.[45]

RV-induced corticosteroid insensitivity may explain why virus-associated asthma exacerbations occur despite optimal anti-inflammatory treatment and require an increase in inhaled or systemic corticosteroid treatment.

New pathways have recently been targeted *in vitro*, in search of new therapeutic options against respiratory viruses most commonly involved in human respiratory infections, including virus-associated exacerbations of obstructive lung diseases. Kinases are implicated in signal transduction pathways involved in steroid responsiveness. Thus, some of the novel compounds focus on them. These compounds, called narrow spectrum kinase inhibitors, are RV568 and RV1088, and *in vitro* studies showed that they are able to inhibit both HRV16-induced inflammation and HRV16 replication.[46,47] Moreover, it is demon-

strated that RV1088 significantly increases HRV-induced IFN-β and IFN-λ mRNA induction, which are innate immune responses to viral respiratory infection.[47] These data suggest that RV1088 and RV568 could be new therapies for viral exacerbations of COPD.[46,47]

In vitro models of bacterial infection and interactions with antibiotics

In vitro studies can help clarify relevant clinical questions. For instance, it is known that ventilated patients in the ICU carry a significant risk of developing VAP caused by *Pseudomonas aeruginosa*, but the same *Pseudomonas* rarely causes pneumonia outside the ICU.[28,48] *In vitro* studies demonstrated that catecholamine/stress hormones can stimulate the growth and infectivity of some bacteria.[49] Since these drugs are frequently prescribed in the ICU,[50] these data suggested the hypothesis that inotropes could affect *Pseudomonas* infection.

Catecholamines are potent stimulators of *Pseudomonas* growth (50-fold increases); they increase *Pseudomonas* biofilm formation on endotracheal tubes, enhance *Pseudomonas* toxicity in its interaction with the respiratory epithelium, and facilitate a rapid recovery of *Pseudomonas* from a tobramycin antibiotic challenge.[51] In contrast, the non-catecholamine inotropes vasopressin and phenylephrine have no stimulatory effect on *Pseudomonas aeruginosa*.[51] These results suggest inotropes to be a risk factor for ventilator-associated pneumonia induced by *Pseudomonas aeruginosa* in patients at intensive care units, proposing new therapeutic approaches to severe patients.[51] Thus, even if *in vitro* studies sometimes appear to be distant from clinical needs, they may be able to raise and answer some clinical questions.

Moreover, since animal models closely imitate the characteristics of human respiratory infections but have the important disadvantage of metabolic differences which imply significant discrepancies in pharmacokinetics (PK),[52,53] *in vitro* models can help investigate antibiotic activities.[52,54] Indeed, *in vitro* models are more flexible and adaptable to different conditions from animal models; therefore, they are able to recapitulate *in vivo* drug clearance profiles and the time course of an antimicrobial agent, mimicking human PK.[35,55]

In addition to the inhered limitation of any *in vitro* condition, i.e. the artificial "unnatural" setting, *in vitro* models have specific shortcomings (Table 2), including the risk of contamination of the culture with external bacteria[56] and the need for special conditions, such as a temperature-controlled environment.[55] Indeed, elevated temperature/fever in humans during infection contributes to bactericidal activity, which must be taken into account when designing *in vitro* studies.[57] Since bacteria grow faster *in vitro* than they do in animal models or in human serum,[58] and the antimicrobial activity of some drugs is related to the rate of bacterial growth,[59] growth speed may be a major limitation of *in vitro* models. In addition, *in vitro* models cannot incorporate all variables seen *in vivo*,[60] especially immunological factors, the inoculum effect of respiratory pathogens,[61] and the virulence and metabolic behavior of a pathogen.[52] Therefore, derived pharmacodynamic (PD) parameters cannot be directly transferred to the *in vivo* situation. However, although *in vitro* pharmacodynamic models cannot reproduce all *in vivo* conditions, they provide valuable data for the development and assessment of antimicrobial therapies.[35]

Mathematical models

PK and PD properties of antibiotics have also been analyzed by mathematical models, which define rules that guide drug behavior.[62] Moreover, in order to gain better insights into the dynamics of viral infections, a mathematical model of the *in vitro* dynamics of viral infection has been developed.[63] This model describes the disease as battle between the virus and the immune system, a combat between invading viral particles and the ability of the inflammatory system to answer by producing substances conferring resistance to the virus.[63] Thus, this model includes infection, cell death, interferon production, and the development of resistance.[63]

Mathematical models are also developing as essential tools to understand the transmission and control of contagions, the progression of epidemics, and the roles of public health interventions (Table 3). The so-called SIR model is one of the simplest epidemiological models that describes the progression of an epidemic. It is based upon calculating the proportion of the population in each of the 3 classes (susceptible, infected, and recovered) and upon determining the rates of transition between them.[64] Mathematical models are also able to analyze some complex situations; they examine the possibilities of spreading diseases, taking into consideration various determi-

Table 3. Role of mathematical models*

Pharmacokinetic-pharmacodynamic	PK/PD analyses and PK/PD modeling.[62]
Viral infection dynamics	Infection, cell death, production of interferon and development of resistance.[63]
Progress of an epidemic	Calculating proportion and transition between three classes: susceptible, infected, recovered people.[64]
Guide public health interventions	Pharmaceutical (drugs, vaccines) and non-pharmaceutical (social distancing).[66]

*illustrates the main applications of mathematical models in the field of respiratory infections starting from PK and PD analyses to guide for public health interventions.
PK, pharmacokinetic; PD, pharmacodynamic.

nants of infectious diseases, such as virulence, spatial distances, and risk factors. Finally, mathematical models could be powerful tools to develop prevention and containment strategies for mitigating the severity of a new influenza pandemic, a top global public health priority. These models also attempt to understand and schematize underlying principles of immunization, in order to develop effective vaccination strategies, addressing major public health interventions.[65,66] However, estimates of policy effectiveness will change if characteristics of future pandemic strains differ substantially from those seen in past pandemics.

Integration of different models

Finally, every model, even the theoretical mathematical model, has some limitations; thus, the best results are derived from combining all models and integrating their outcomes.

A good example is the integration of studies regarding interferon-inducible transmembrane proteins (IFITM). It has been documented *in vitro* that IFITM3 restricts the replication of multiple pathogenic viruses, including influenza.[67] Deletion of the IFTM locus leads to increased severity of influenza A virus disease, while restoration of this protein decreases viral infectivity.[67] These results were confirmed by animal models. Indeed, mice lacking IFITM3 display fulminant viral pneumonia when challenged with a normally low- pathogenicity influenza virus.[68] The role of IFITM3 in the protection from influenza was also demonstrated in humans. Indeed, severe infections requiring hospitalization occurr in patients bearing a IFITM3 allele called "C", which has functional defects and causes reduced influenza restriction.[68] Taken together, these data indicate that the action of a single intrinsic immune effector, IFITM3, profoundly alters the course of influenza virus infection.[68]

Other well-defined examples of different model integration are studies on Th1/Th2 ratio in asthmatics. An important *in vitro* study, based on primary bronchial epithelial cells and alveolar macrophages, demonstrated impaired interferon production by RV in asthmatics.[41] In the human model of RV infection, this deficit is involved in reduced ability of asthmatics to eliminate viruses, as revealed by relationship between viral load and severity of RV-induced asthma exacerbations.[41] The impaired interferon production characterizes not only asthma, but also other conditions, such as atopy, and it is also present in children as demonstrated *ex vivo* in bronchial biopsy specimens and epithelial cells obtained from children undergoing bronchoscopy.[69] Similarities in antiviral responses among different conditions characterized by Th2/Th1 imbalance is associated with similar pathologic findings. Indeed, an *ex vivo* study on bronchial biopsies, obtained from children undergoing bronchoscopy, demonstrated that epithelial damage and basement membrane thickening, which are typical of adult asthma, are also observed in childhood asthma.[70] Moreover, airway eosinophilia and angiogenesis appear even in atopic non-asthmatic children.[70]

The strength of these data are derived from accumulating evidence, *in vivo*, *ex vivo*, and *in vitro*. The integration of information derived from different settings provides additive, complementary, reciprocally potentiating pieces of evidence at different levels of system biology, supporting the robustness of the overall structure of the hypotheses. When *in vitro* results are confirmed in animal and human models, and *vice-versa*, evidence supports each other, evaluating different aspects and mechanisms. *In vitro* studies permit us to evaluate in detail the role of different molecules without confounding factors; animal studies permit us to assess aspects closer to the real condition; human models explore real-life and clinical conditions; mathematical models analyze all information for obtaining general rules to predict the progression of epidemics and to plan public health interventions. Thus, different models are complementary in their attempt to clarify disease pathogenesis, and they could inform new perspectives for intervention (pharmacological and non- pharmacological) strategies.

CONCLUSION

Human disease models are the closest approximations to real-life conditions but difficult to realize; therefore, animal models have been developed. However, animals are similar, but not exact models of humans; therefore, these models may have important differences. Thus, *in vitro* models have been widely used, and their results have been further elaborated in order to obtain theoretical schemes by mathematical models. Nevertheless, every model, even the abstract mathematical model, has some limitations; thus, the best results can be obtained by examining all models collectively and integrating their outcomes.

REFERENCES

1. DeVincenzo JP, Wilkinson T, Vaishnaw A, Cehelsky J, Meyers R, Nochur S, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. Am J Respir Crit Care Med 2010;182:1305-14.
2. You SY, Jwa HJ, Yang EA, Kil HR, Lee JH. Effects of methylprednisolone pulse therapy on refractory Mycoplasma pneumoniae pneumonia in children. Allergy Asthma Immunol Res 2014;6:22-6.
3. Mallia P, Contoli M, Caramori G, Pandit A, Johnston SL, Papi A. Exacerbations of asthma and chronic obstructive pulmonary disease (COPD): focus on virus induced exacerbations. Curr Pharm Des 2007;13:73-97.
4. Johnston SL, Pattemore PK, Sanderson G, Smith S, Lampe F, Josephs L, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. BMJ 1995;310:1225-9.
5. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993;307:982-6.
6. Papadopoulos NG, Johnston SL. Rhinoviruses as pathogens of the lower respiratory tract. Can Respir J 2000;7:409-14.
7. Papadopoulos NG, Psarras S. Rhinoviruses in the pathogenesis of

asthma. Curr Allergy Asthma Rep 2003;3:137-45.
8. Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. Proc Natl Acad Sci U S A 2008;105:13562-7.
9. Fraenkel DJ, Bardin PG, Sanderson G, Lampe F, Johnston SL, Holgate ST. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. Am J Respir Crit Care Med 1995; 151:879-86.
10. Zhu J, Message SD, Qiu Y, Mallia P, Kebadze T, Contoli M, et al. Airway inflammation and illness severity in response to experimental rhinovirus infection in asthma. Chest 2014;145:1219-29.
11. Mallia P, Message SD, Gielen V, Contoli M, Gray K, Kebadze T, et al. Experimental rhinovirus infection as a human model of chronic obstructive pulmonary disease exacerbation. Am J Respir Crit Care Med 2011;183:734-42.
12. Bem RA, Domachowske JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. Am J Physiol Lung Cell Mol Physiol 2011;301:L148-56.
13. Acosta A, Norazmi MN, Hernandez-Pando R, Alvarez N, Borrero R, Infante JF, et al. The importance of animal models in tuberculosis vaccine development. Malays J Med Sci 2011;18:5-12.
14. Bartlett NW, Walton RP, Edwards MR, Aniscenko J, Caramori G, Zhu J, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med 2008;14:199-204.
15. Nagarkar DR, Bowman ER, Schneider D, Wang Q, Shim J, Zhao Y, et al. Rhinovirus infection of allergen-sensitized and -challenged mice induces eotaxin release from functionally polarized macrophages. J Immunol 2010;185:2525-35.
16. Hong JY, Chung Y, Steenrod J, Chen Q, Lei J, Comstock AT, et al. Macrophage activation state determines the response to rhinovirus infection in a mouse model of allergic asthma. Respir Res 2014; 15:63.
17. Mori H, Parker NS, Rodrigues D, Hulland K, Chappell D, Hincks JS, et al. Differences in respiratory syncytial virus and influenza infection in a house-dust-mite-induced asthma mouse model: consequences for steroid sensitivity. Clin Sci (Lond) 2013;125:565-74.
18. Clarke DL, Davis NH, Majithiya JB, Piper SC, Lewis A, Sleeman MA, et al. Development of a mouse model mimicking key aspects of a viral asthma exacerbation. Clin Sci (Lond) 2014;126:567-80.
19. Wright JL, Cosio M, Churg A. Animal models of chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 2008; 295:L1-15.
20. Beasley V, Joshi PV, Singanayagam A, Molyneaux PL, Johnston SL, Mallia P. Lung microbiology and exacerbations in COPD. Int J Chron Obstruct Pulmon Dis 2012;7:555-69.
21. Churg A, Sin DD, Wright JL. Everything prevents emphysema: are animal models of cigarette smoke-induced chronic obstructive pulmonary disease any use? Am J Respir Cell Mol Biol 2011;45: 1111-5.
22. Sajjan U, Ganesan S, Comstock AT, Shim J, Wang Q, Nagarkar DR, et al. Elastase- and LPS-exposed mice display altered responses to rhinovirus infection. Am J Physiol Lung Cell Mol Physiol 2009;297: L931-44.
23. Meshi B, Vitalis TZ, Ionescu D, Elliott WM, Liu C, Wang XD, et al. Emphysematous lung destruction by cigarette smoke. The effects of latent adenoviral infection on the lung inflammatory response. Am J Respir Cell Mol Biol 2002;26:52-7.
24. Drannik AG, Pouladi MA, Robbins CS, Goncharova SI, Kianpour S, Stämpfli MR. Impact of cigarette smoke on clearance and inflammation after Pseudomonas aeruginosa infection. Am J Respir Crit Care Med 2004;170:1164-71.
25. Pang B, Hong W, West-Barnette SL, Kock ND, Swords WE. Diminished ICAM-1 expression and impaired pulmonary clearance of nontypeable Haemophilus influenzae in a mouse model of chronic obstructive pulmonary disease/emphysema. Infect Immun 2008;76:4959-67.
26. Keller CE, Elliott TB, Bentzel DE, Mog SR, Shoemaker MO, Knudson GB. Susceptibility of irradiated B6D2F1/J mice to Klebsiella pneumoniae administered intratracheally: a pulmonary infection model in an immunocompromised host. Comp Med 2003;53:397-403.
27. Marquette CH, Wermert D, Wallet F, Copin MC, Tonnel AB. Characterization of an animal model of ventilator-acquired pneumonia. Chest 1999;115:200-9.
28. Garau J, Gomez L. Pseudomonas aeruginosa pneumonia. Curr Opin Infect Dis 2003;16:135-43.
29. Luna CM, Baquero S, Gando S, Patrón JR, Morato JG, Sibila O, et al. Experimental severe Pseudomonas aeruginosa pneumonia and antibiotic therapy in piglets receiving mechanical ventilation. Chest 2007;132:523-31.
30. Shultz LD, Brehm MA, Bavari S, Greiner DL. Humanized mice as a preclinical tool for infectious disease and biomedical research. Ann N Y Acad Sci 2011;1245:50-4.
31. Dupuit F, Gaillard D, Hinnrasky J, Mongodin E, de Bentzmann S, Copreni E, et al. Differentiated and functional human airway epithelium regeneration in tracheal xenografts. Am J Physiol Lung Cell Mol Physiol 2000;278:L165-76.
32. Mongodin E, Bajolet O, Cutrona J, Bonnet N, Dupuit F, Puchelle E, et al. Fibronectin-binding proteins of Staphylococcus aureus are involved in adherence to human airway epithelium. Infect Immun 2002;70:620-30.
33. Brehm MA, Cuthbert A, Yang C, Miller DM, DiIorio P, Laning J, et al. Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation. Clin Immunol 2010;135:84-98.
34. Strowig T, Rongvaux A, Rathinam C, Takizawa H, Borsotti C, Philbrick W, et al. Transgenic expression of human signal regulatory protein alpha in Rag2-/-gamma(c)-/- mice improves engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci U S A 2011;108:13218-23.
35. White RL. What in vitro models of infection can and cannot do. Pharmacotherapy 2001;21:292S-301S.
36. Terajima M, Yamaya M, Sekizawa K, Okinaga S, Suzuki T, Yamada N, et al. Rhinovirus infection of primary cultures of human tracheal epithelium: role of ICAM-1 and IL-1beta. Am J Physiol 1997;273: L749-59.
37. Subauste MC, Jacoby DB, Richards SM, Proud D. Infection of a human respiratory epithelial cell line with rhinovirus. Induction of cytokine release and modulation of susceptibility to infection by cytokine exposure. J Clin Invest 1995;96:549-57.
38. Spannhake EW, Reddy SP, Jacoby DB, Yu XY, Saatian B, Tian J. Synergism between rhinovirus infection and oxidant pollutant exposure enhances airway epithelial cell cytokine production. Environ Health Perspect 2002;110:665-70.
39. Edwards MR, Johnson MW, Johnston SL. Combination therapy: synergistic suppression of virus-induced chemokines in airway ep-

ithelial cells. Am J Respir Cell Mol Biol 2006;34:616-24.
40. Papi A, Papadopoulos NG, Degitz K, Holgate ST, Johnston SL. Corticosteroids inhibit rhinovirus-induced intercellular adhesion molecule-1 up-regulation and promoter activation on respiratory epithelial cells. J Allergy Clin Immunol 2000;105:318-26.
41. Contoli M, Message SD, Laza-Stanca V, Edwards MR, Wark PA, Bartlett NW, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. Nat Med 2006;12:1023-6.
42. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. Thorax 2002;57:328-32.
43. Mathur SK, Fichtinger PS, Kelly JT, Lee WM, Gern JE, Jarjour NN. Interaction between allergy and innate immunity: model for eosinophil regulation of epithelial cell interferon expression. Ann Allergy Asthma Immunol 2013;111:25-31.
44. Shin SH, Ye MK, Kim JK. Effects of fungi and eosinophils on mucin gene expression in rhinovirus-infected nasal epithelial cells. Allergy Asthma Immunol Res 2014;6:149-55.
45. Papi A, Contoli M, Adcock IM, Bellettato C, Padovani A, Casolari P, et al. Rhinovirus infection causes steroid resistance in airway epithelium through nuclear factor κB and c-Jun N-terminal kinase activation. J Allergy Clin Immunol 2013;132:1075-1085.e6.
46. Contoli M, Ito K, Papi A. Effects of RV568, a narrow spectrum kinase inhibitor, on HRV16 replication and cytokine production in airway epithelial cells obtained from COPD patients. American Thoracic Society International Conference Abstracts of American Thoracic Society 2011 International Conference; 2011 May 13-18; Denver. New York (NY): American Thoracic Society; 2011. A2766 p.
47. Contoli M, Ito K, Papi A. Effects of RV1088, a narrow spectrum kinase inhibitor, on HRV16 replication and cytokine production in airway epithelial cells obtained from COPD patients. American Thoracic Society International Conference Abstracts of American Thoracic Society 2011 International Conference; 2011 May 13-18; Denver. New York (NY): American Thoracic Society; 2011. A3368 p.
48. Morehead RS, Pinto SJ. Ventilator-associated pneumonia. Arch Intern Med 2000;160:1926-36.
49. Lyte M, Ernst S. Catecholamine induced growth of gram negative bacteria. Life Sci 1992;50:203-12.
50. Smythe MA, Melendy S, Jahns B, Dmuchowski C. An exploratory analysis of medication utilization in a medical intensive care unit. Crit Care Med 1993;21:1319-23.
51. Freestone PP, Hirst RA, Sandrini SM, Sharaff F, Fry H, Hyman S, et al. Pseudomonas aeruginosa-catecholamine inotrope interactions: a contributory factor in the development of ventilator-associated pneumonia? Chest 2012;142:1200-10.
52. European Agency for the Evaluation of Medicinal Products (GB). CPMP/EWP/2655/99. Points to Consider on Pharmacokinetics and Pharmacodynamics in the Development of Antibacterial Medicinal Products. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003420.pdf.
53. Takahashi M, Washio T, Suzuki N, Igeta K, Yamashita S. The species differences of intestinal drug absorption and first-pass metabolism between cynomolgus monkeys and humans. J Pharm Sci 2009;98:4343-53.
54. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis 1998;26:1-10.
55. Gloede J, Scheerans C, Derendorf H, Kloft C. In vitro pharmacodynamic models to determine the effect of antibacterial drugs. J Antimicrob Chemother 2010;65:186-201.
56. Wang L, Wismer MK, Racine F, Conway D, Giacobbe RA, Berejnaia O, et al. Development of an integrated semi-automated system for in vitro pharmacodynamic modelling. J Antimicrob Chemother 2008;62:1070-7.
57. Mackowiak PA, Marling-Cason M, Cohen RL. Effects of temperature on antimicrobial susceptibility of bacteria. J Infect Dis 1982;145:550-3.
58. Brown MR, Williams P. Influence of substrate limitation and growth phase on sensitivity to antimicrobial agents. J Antimicrob Chemother 1985;15 Suppl A:7-14.
59. Tuomanen E, Cozens R, Tosch W, Zak O, Tomasz A. The rate of killing of Escherichia coli by beta-lactam antibiotics is strictly proportional to the rate of bacterial growth. J Gen Microbiol 1986;132:1297-304.
60. Lorian V. In vitro simulation of in vivo conditions: physical state of the culture medium. J Clin Microbiol 1989;27:2403-6.
61. Brook I. Inoculum effect. Rev Infect Dis 1989;11:361-8.
62. Czock D, Keller F. Mechanism-based pharmacokinetic-pharmacodynamic modeling of antimicrobial drug effects. J Pharmacokinet Pharmacodyn 2007;34:727-51.
63. Getto P, Kimmel M, Marciniak-Czochra A. Modelling and analysis of dynamics of viral infection of cells and of interferon resistance. J Math Anal Appl 2008;344:821-50.
64. Keeling MJ, Danon L. Mathematical modelling of infectious diseases. Br Med Bull 2009;92:33-42.
65. Moghadas SM. Gaining insights into human viral diseases through mathematics. Eur J Epidemiol 2006;21:337-42.
66. Yang W, Sun C, Arino J. Global analysis for a general epidemiological model with vaccination and varying population. J Math Anal Appl 2010;372:208-23.
67. Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 2009;139:1243-54.
68. Everitt AR, Clare S, Pertel T, John SP, Wash RS, Smith SE, et al. IFITM3 restricts the morbidity and mortality associated with influenza. Nature 2012;484:519-23.
69. Baraldo S, Contoli M, Bazzan E, Turato G, Padovani A, Marku B, et al. Deficient antiviral immune responses in childhood: distinct roles of atopy and asthma. J Allergy Clin Immunol 2012;130:1307-14.
70. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, et al. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med 2006;174:975-81.